Original article:

Toxicological Studies of popular eye cosmetic used world wide

Tehmina Sohail[1], Atiq-ur-Rahman[2],. Hina Imran[3], Zahra Yaqeen[4], Nudrat Fatima[5], Zakir-ur-Rehman[6], Khula Shireen[7]

Abstract:

Background and Objective: Present research work was designed and conducted to verify the safe use of kajal, a popular eye cosmetic, which is widely used to beautify eyes throughout the world especially in South Asia and Middle East region. ***Material and Method*:** The toxicological studies were conducted in experimental animals for a period of 90 days to clarify misleading thoughts associated with its long term use including lead toxicity. The study was carried out in Albino rats of Wister strain and New Zealand White rabbits. Hashmi kajal dibya (net weight 4.25g), an eye cosmetic manufactured by M/s A.Q. and company international Pakistan was used as source for study. The ingredients claimed by the manufacturer are zinc oxide, wax, *cinnamonum camphora*, processed carbon black and clarified butter. ***Result:*** The elemental analysis resulted in presence of zinc 9.56%, lead 0.09% and total ash 41.01%, while sulfur, antimony, mercury and arsenic were not detected in the sample. The sub-chronic toxicological studies revealed that no toxicological effects were found in experimental animals. All the animals of test and control groups exhibited normal physiological activities and an increase in body weights. Lead and Zinc levels remained constant throughout the experimental period and no mortality was recorded. Furthermore, liver and kidney function tests were normal, indicating non-toxic effect of the kajal on vital organs. ***Conclusion:*** So it can be assumed that this eye cosmetic is non-toxic and can be used safely in humans.

Keywords: Toxicity; Eye cosmetic; elemental analysis; blood biochemistry

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Introduction:

Ornamented eyes are ubiquitous in ancient and modern world and their depiction remains consistent through out the history.Impecable dark lined eyes add beauty and charm to personality.History of ancient art of Egypt, North Africa and Mrocco depicts that hardly a portrait exist without an impeccably dark out lined eyes with kohl[1,2,3] Kajal or Kohl has been used for centuries worldwide especially in eastern cultures for beautification of eyes. It is claimed to keep the eyes clean and protect the eyes from various forms of dirt. Further the population believes that it is a remedy for infected, inflamed and painful eyes. Freshly prepared herbal Kajal is also applied to the eyes of new born babies. In rural areas, some people use it to protect the glance of evil eye while others to keep the eyes clean[4]. In spite of studies on the extent use and toxic effects of eye cosmetics due to lead and other ingredients used in composition of these preparations[5]. Eye cosmetics hold great potential and interest in use of kohl or kajal has been growing as an eye cosmetic to keep the eyes clean from atmospheric pollution, [6,7,8]. In view of the above facts, this study was conducted on research animals,

1. Tehmina Sohail
2. Atiq-ur-Rahman
3. Hina Imran
4. Zahra Yaqeen
5. Nudrat Fatima
6. Zakir-ur-Rehman
7. Khula Shireen

Pharmaceutical Research Centre, PCSIR Laboratories Complex, Karachi, Pakistan

Correspondence to: Tehmina Sohail, SSO, Pharmaceutical Research Centre, PCSIR Laboratories Complex, Karachi, Pakistan. Email: d.tehmina@yahoo.com

in order to confirm or contradict the previous data, which is insufficient and confusing for its use as an eye cosmetic.

Material and Method

Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade (Merck, Germany). Triple distilled water was used where required. Glassware used was of standard quality (pyrex) and was properly sterilized.

Composition of Kajal

According to manufacturer the kajal contains Zinc oxide,*Cinnamomum camphora*, some herbs clarified butter, oil, carbon black and waxes.

Animals

Albino rats of Wistar strain (weight between 145g-200g) and New Zealand White rabbits (weight between 1.25kg to 1.75kg) of either sex were used in the study. The animals were procured from Animal House of PCSIR Labs Complex, Karachi and were fed with standard laboratory animal diet. The study protocol was approved by the Ethical Committee, PCSIR before starting the experiment. .

Elemental Analysis

Elemental analysis was performed according to the standard method using atomic absorption spectrophotometer (Hitachi model Z-5000) equipped with Zeeman Background Corrector and Data Processor[9].

Toxicological Studies

Both acute and chronic toxicity studies were carried out in experimental animals i.e. rats and rabbits. The animals were observed during acute oral toxicity test for two weeks and for a period of ninety days during sub-chronic toxicity study.

Acute Oral Toxicity Test

Acute oral toxicity test was performed according to the standard reported method in albino rats of Wistar strain weighing between 145g to 200g. The sample was dissolved in olive oil to give a concentration of 100µg/ml. Animals were divide into three groups (n=10) each group comprising of 5 male and 5 female rats. Group I was given test sample in a single dose, group II was given test sample in double dose and group III was given distilled water only. The cages of animals were marked with their respective doses and were observed strictly for the first six hours and then for the period of 72 hours. Daily observation on general health, growth, gross physical and behavioral activities and also the mortality if any was noted and recorded up to two weeks[10,11].

Sub Chronic Toxicological Study

Animals, i.e. New Zealand White rabbits of either sex weighing between 1.5-2.0 kg were divided into three groups, each group comprised of 10 animals (5 male and 5 female). Group 1 received single application of Kajal in a dose of about 7-8 µg/day through an applicator provided with the sample, group 2 received double application of test sample in a dose of about 14-16 µg/ day, while group 3 served as control group and received no application. The animals were provided with water and food comprising of alfa alfa, black grams and carrots *ad libitum*. Cages were marked and observed strictly. During observation the parameters like gross physical and behavioral changes, morbidity and mortality were noted. Animal body weights and blood biochemistry was noted at baseline (0 day), after 45 days and after completion of 90 days study period.

Biochemical Analysis

For biochemical analysis, blood was collected from marginal vein of 03 male and 03 female animals from each group at 0,45 and 90 days intervals and subjected to liver and kidney function tests. The blood sample was also analyzed for lead and zinc content by Atomic Absorption Spectrophotometer[12].

Histopathological Study

Animals were slaughtered at the end of the study and histopathological assessment was carried out to find out changes, if any, after 90 day application of kajal.Vital organs i.e. liver, spleen and kidneys were removed from each animal of both the test groups on the 0, 45 and 90th day after sacrificing the animals to find out any drug related histopathological changes. Abdominal cavities of the animals were opened and liver, spleen and kidney were excised out and kept on blotting paper. Tissues were then transferred to glass bottles containing 10% buffered isotonic formalin solution. Solution was changed within 24 hours to clear any residual blood.

Tissues were passed through different solutions for dehydration, clearing and impregnation. They were then embedded into paraffin to form blocks. Approximately 5µm sections were cut from the tissue blocks, fixed on glass slides, stained with hematoxylin and eosin and then sealed with cover slips. Liver specimens were stained with PAT, Masson's trichrome, reticulin and Prussian blue. Renal tissues were stained with PAS and silver strains.

Slides thus prepared were examined under the light microscope for evidence of any histological change. Liver specimens were evaluated for necrosis,

inflammation and any abnormal accumulation. Splenic tissues were evaluated for secondary germinal centers and any increase in cordal macrophages or pigment. Renal glomeruli, tubules, interstitium and blood vessels were evaluated for any change.

Statistical analysis

The values were calculated as mean ± SD. All the data was analyzed statistically by student *t* test. The *p* values at ≤ 0.01 level were considered significant[13].

Ethical Approval: This study was approved by the Pharmaceutical Research Centre, PCSIR Laboratories Complex, Karachi, Pakistan.

Results

Elemental analysis of kajil revealed the presence of Lead (0.33ug), Zinc(1.58ug),Antimony (0.0006ug) and Sulpher (0.11ug) as showed in table 1.Mercury and Arsenic were not detected in the kajal sample. Biochemical analysis of blood at 0,45 and 90th days indicated that there was no change observed in blood biochemistry in any group. All kidney and liver functions test(Total Bilirubin, Albumin and creatinine levels) were normal. Elemental analysis of the blood samples drawn at 0,45,90th day of observation period revealed the presence of zinc and lead while sulphur and antimony were not detected although they were present in the original sample. The levels elements remained constant through out the observation period i.e the level of elements which were present on the day remained approximately constant through out 90 days as showed in table 3 4, 5.Autopsy and histopathological findings of all vital organs i.e heart, liver,spleen and kidney indicated that there were no abnormal histological lesions (necrosis, inflammation in liver pigmentation and increase in cordal macrophages in spleen) present.

Table 1: Elemental analysis of sample

S.No	Element analyzed	% detected	Quantity present in single application
1	Zinc	9.56	1.53µg
2	Sulfur	Not detected	Nil
3	Lead	0.09	0.01µg
4	Antimony	Not detected	Nil
5	Mercury	Not detected	Nil
6	Arsenic	Not detected	Nil

Table 2: Biochemical Analysis of rabbits' blood at baseline (0 Day)

S#	Parameter	Group 1 Male	Group 1 Female	Group 2 Male	Group 2 Female	Group 3 Male	Group 3 Female	F; p value
1	Body weight	1468 ± 2.5	1288 ±1.6	1253 ± 1.5	1356 ± 4.2	1470 ± 3.5	1454 ±3.2	0.005; 0.100*
2	Zinc (µg/dl)	2.4 ± 0.046	0.78 ± 0.15	1.096 ±0.10	2.246±0.025	0.953±0.025	1.2 ±0.043	65.7; 0.00
3	Lead (µg/dl)	0.19±0.005	0.196±0.015	0.246±0.02	0.153±0.015	0.203±0.015	0.29±0.046	0.325; 0.893*
4	T. Bilirubin	0.7±0.02	0.503±0.015	0.696±0.015	0.65±0.00	0.683±0.005	0.596±0.015	142.3; 0.00
5	SGPT	25.33±1.527	37.66±0.577	36.33±1.527	28.0±1.00	35.33±0.577	29.0±2.00	76.3; 0.00
6	Alk. PO_4	112±3.00	67.66±0.577	173±4.582	96.33±3.511	148±2.00	80.66±0.577	1047.78; 0.00
7	SGOT	81.66±1.527	37.0±0.00	71.66±1.527	37.33±0.577	52.66±2.516	42.66±1.527	779.3; 0.00
8	Urea	64.00±3.00	76.00±1.00	76.00±0.00	76.66±0.577	93.00±3.00	71.00±2.00	114.3; 0.00
9	Creatinine	1.266±0.152	1.266±0.115	1.767±0.057	1.133±0.057	1.50±0.100	1.20±0.00	31.793; 0.00
10	T. protein	9.50±0.20	7.10±0.100	8.16±0.351	6.066±0.513	7.766±0.057	8.033±0.153	82.856;0.00
11	Albumin	5.70±0.00	4.20±0.02	5.00±0.00	3.933±0.057	3.866±0.057	4.766±0.152	507.1; 0.00

All data is presented as mean ± SD; *p* value≤ 0.01; *= non-significant *p* value

Table 3: Biochemical Analysis of rabbits' blood at 45th Day

S#	Parameters	Group1 Male	Group 1 Female	Group 2 Male	Group 2 Female	Group3 Male	Group 3 Female	F;p value
1	Body weight	1646 ± 2.3	1400 ±2.1	1392 ± 1.2	1422 ± 4.1	1553 ± 3.6	1486 ± 2.8	0.006; 1.00*
2	Zinc (µg/dl)	2.66±0.070	1.00±0.199	1.14±0.066	1.18±0.065	1.39±0.055	1.196±0.025	201.0; 0.00
3	Lead (µg/dl)	0.186±0.015	0.206±0.015	0.193±0.005	0.176±0.025	0.193±0.015	0.27±0.005	25.37; 0.00
4	T. Bilirubin	0.796±0.015	0.750±0.010	0.646±0.208	0.653±0.005	0.503±0.015	0.626±0.005	7.197; 0.00
5	SGPT	29.0±1.00	24.00±0.00	26.33±1.527	27.66±0.577	35.33±1.154	38.00±0.00	178.1; 0.00
6	Alk.PO_4	100.6±0.577	54.33±2.516	91.66±3.055	57.66±1.527	127.6±3.511	47.66±1.527	1322.4; 0.00
7	SGOT	65.33±1.527	56.66±1.527	69.33±0.577	54.66±1.527	41.00±1.00	38.66±2.516	211.42; 0.00
8	Urea	42.33±0.577	82.66±2.516	47.00±1.732	55.33±0.577	62.66±2.516	62.00±2.00	300.1; 0.00
9	Creatinine	1.20±0.100	0.966±0.057	1.366±0.152	1.100±0.100	0.966±0.057	1.133±0.057	13.044; 0.00
10	T. protein	7.066±0.057	6.10±1.087	7.233±0.251	6.266±0.152	6.133±0.152	7.766±0.152	10.943; 0.00
11	Albumin	5.133±0.152	4.10±0.00	5.33±0.351	4.266±0.152	4.333±0.152	4.466±0.057	38.85; 0.00

All data is presented as mean ± SD; *p* value≤ 0.001; *= non-significant *p* value

Table 4: Biochemical Analysis of rabbits' blood at 90th Day

S#	Parameters	Group 1 Male	Group 1 Female	Group 2 Male	Group 2 Female	Group3 Male	Group 3 Female	F; p value
1	Body weight	1667 ± 1.5	1505 ± 2.7	1412 ± 1.2	1452 ± 3.2	1577 ± 3.8	1523 ± 2.2	0.006;1.00*
2	Zinc (µg/dl)	0.826±0.015	0.84±0.01	1.146±0.047	1.05±0.132	1.3±0.20	0.94±0.045	16.607; 0.00
3	Lead (µg/dl)	0.186±0.005	0.18±0.01	0.19±0.01	0.16±0.01	0.16±0	0.286±0.015	120.5;0.00
4	T. Bilirubin	0.696±0.015	0.703±0.045	0.646±0.005	0.750±0.026	0.646±0.015	0.700±0.010	14.243; 0.00
5	SGPT	43.66±1.527	31.66±2.081	38.33±1.527	55.66±2.309	61.66±2.081	60.67±5.131	104.4;0.00
6	Alk. PO_4	89.66±2.516	99.00±3.00	101.3±1.527	37.33±8.621	72.33±4.163	57.33±2.516	166.6;0.00
7	SGOT	94.00±3.605	33.00±4.582	85.00±2.645	52.33±3.511	69.33±2.516	47.33±2.516	249.4;0.00
8	Urea	42.00±2.00	76.33±0.577	64.33±1.527	53.66±3.511	74.66±2.516	88.66±3.511	226.9;0.00
9	Creatinine	1.033±0.152	1.193±0.040	1.113±0.032	1.286±0.015	0.956±0.140	1.190±0.045	1.396;0.261*
10	T. protein	8.366±0.152	14.50±0.458	10.03±0.251	10.96±0.152	7.50±0.300	11.56±0.351	350.7;0.00
11	Albumin	4.816±0.076	4.60±0.160	5.193±0.190	4.326±0.231	5.380±0.098	3.403±0.205	20.17;0.00

All data is presented as mean ± SD; *p* value≤ 0.001; *= non-significant *p* value

Discussion

The present study was conducted to assess the local application/use of kajal and its effect if any after application in the eye and to see subsequent effect on vital organs if any. As mentioned in some other studies that kohl application increases blood lead concentration is not proved practically but a theoretical assumption only[14].

Long-term application of kajal in experimental animal(rabbits) in concentration up to 16µg/eye daily did not produce any untoward effects like redness, swelling, discharge, irritation and chemosis which documents its safety, rather its prolong use imparted beneficial effects on eyes. It cleaned the eyes and made them bright and shiny. These beneficial effects can be attributed to the ingredients as well as oily nature, which has absorptive action, produces astringent effects after its application on upper and lower lids of eyes and it accumulates dust and dirt entering into the eyes. The composition of eye is such that it can absorb only hydrophilic compounds[15] so there is no chance of absorption of kajal through eyes as kajal which has been used in this study is non hydrophilic. Further more conjunctiva covers most of the ocular surface and have great permeability for hydrophilic compounds[16]. When kajal is applied in eyes being oily in nature does not absorbed either through conjunctiva or cornea but it only cleans the eyes from dirt. The presence of zinc is essential for maintaining normal ocular function. It is reported that deficiency of zinc results in cataract[17,18]. The herb, *Cinnamonum camphora*, added in preparation is antiseptic and is used frequently for soothing and pain relieving purpose. This herb plays role in visual improvement as reported by some researchers[19]. Essential oils of *Cinnamonum camphora* has excellent biological properties like antibacterial, antifungal and antioxidant[20] which verified the traditional use of kajal in treatment of eye infections. Similarly changes attributed to lead toxicity were also not observed in experimental animals. Lead in high concentration produces neurological, gastro-intestinal, renal and hematopoietic changes. The present study reveals that no such effects take place even after prolonged use and all liver and kidney functions tests were found normal. There was no difference in body weight, health and behavior of animals of test and control groups. No mortality was recorded during the experimental period. Zinc and Lead level was remained normal and within limits throughout the experimental period.

Conclusion

Keeping in view of the results of this study, it is concluded that the continuous use of kajal in eyes is neither toxic nor produce ill effects on general health of an individual and therefore, it is safe to be used as an eye cosmetic in human.

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Authors' Contributions: Data gathering and idea owner of this study: Tehmina Sohail

Study design: Tehmina Sohail, Atiq-ur-Rahman

Data gathering: Tehmina Sohail, Atiq-ur-Rahman, Hina Imran

Writing and submitting manuscript: Tehmina Sohail, Zahra Yaqeen, Nudrat Fatima

Editing and approval of final draft: Tehmina Sohail, Zakir-ur-Rehman, Khula Shireen

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